THE REMINERALIZATION POTENTIAL OF CPP-ACP (GC TOOTH MOUSSE) AND TCP WITH 0.21% W/W SODIUM FLUORIDE ANTI-CAVITY PASTE (CLINPRO TOOTH CRÈME) ON ARTIFICIAL CARIES – LIKE SUBSURFACE LESIONS IN PRIMARY AND PERMANENT TEETH – AN IN VITRO STUDY

Dissertation submitted to

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In partial fulfillment for the degree of

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BRANCH VIII

PEDODONTICS AND PREVENTIVE DENTISTRY

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CERTIFICATE

This is to certify that this dissertation titled **"THE REMINERALIZATION POTENTIAL OF CPP-ACP (GC TOOTH MOUSSE) AND TCP WITH 0.21% W/W SODIUM FLUORIDE ANTI-CAVITY PASTE (CLINPRO TOOTH CRÈME) ON ARTIFICIAL CARIES – LIKE SUBSURFACE LESIONS IN PRIMARY AND PERMANENT TEETH – AN IN VITRO STUDY"** is a bonafide record of work done by **Dr. R. ARUN PRASAD,** under my guidance during his postgraduate study period between 2008–2011.

This dissertation is submitted to **THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY,** in partial fulfillment for the degree of **Master of Dental Surgery** in Branch VIII –Pedodontics and Preventive Dentistry.
It has not been submitted (partially or fully) for the award of any other degree or diploma.

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INTRODUCTION

Dental caries can be defined "as the localized destruction of tissues of the tooth by bacterial fermentation of dietary carbohydrates". Teeth are covered by dental plaque that contains bacteria. Some of the bacteria, including mutans streptococci and lactobacilli, produce acids when they metabolize fermentable carbohydrates such as glucose, sucrose, fructose, or cooked starch. The acids produced by this metabolism enter into the pores of sound tooth enamel or exposed dentin and dissolve minerals in the tooth structure. This causes a loss of calcium and phosphate from the tooth, resulting in demineralization. Demineralization is first visible as a "**white spot lesion**" on the surface of the tooth enamel.

White spot lesions are an early sign of tooth decay that, if left untreated, will progress to frank caries lesions. Treatment of these demineralized areas can stop progression and reverse the decay process through remineralization. Various methods are available for reversal of these white spot lesions. Fluoride is one of the preventive agents that have been categorized as strongly cariostatic and can be used in various forms. It has both systemic and topical action[10, 11]. It can be used alone or in combination with other remineralizing agents[9]. The other materials with new Calcium

technology that are commercially available are GC tooth mousse (CPP-ACP)[2,28] and Clinpro tooth crème (TCP with 0.21% w/w Sodium Fluoride).

GC tooth mousse contains a promising calcium technology (casein phosphopeptide-amorphous calcium phosphate, CPP-ACP, Recaldent) [34]. At 1.0% w/v, CPP stabilize 60 mmol/L CaCl2 and 36 mmol/L sodium phosphate at pH 7.0 through the formation of colloidal casein-phosphopeptide calcium-phosphate complexes (CPP-ACP). CPP-ACP has been found to increase the levels of calcium and phosphate in plaque up to five-fold in human in situ caries models in short-term mouthwash studies[36]. Reynolds proposed the mechanism of its anticariogenicity to its action as a calcium-phosphate reservoir, buffering the activities of free calcium and phosphate ions in the plaque fluid helping to maintain a state of supersaturation with respect to enamel mineral, thereby depressing enamel demineralization and enhancing remineralization, however the majority of the calcium phosphate stabilized by the CPP is in the form of ACP bound to the phosphopeptides (Reynolds et al., 1995)[21,33,35].

Clarkson et al. (1991) suggested that the presence of soluble phosphoproteins from dentin inhibited remineralization of root caries. The release of the soluble phosphoprotein into the remineralizing solution presumably complexed the calcium phosphate of the solution, reducing the activities of the calcium and phosphate ions such that remineralization was

inhibited. It is therefore unclear whether CPP-stabilized calcium phosphate ions are available to prevent enamel demineralization and promote remineralization.[6]

Recently the evolution of a new prospective calcium system tricalcium phosphate with 0.21% w/w Sodium Fluoride (Clinpro Tooth Creme - a form functionalized calcium phosphate) was reported[19,20]. The calcium and phosphate which are manifested in the saliva and plaque, when combined with fluoride of this prospective material may also interact with weakened enamel to help boost fluoride's remineralizing benefits.

Clinpro Tooth Creme contains 0.21% w/w sodium fluoride and an innovative tri-calcium phosphate ingredient which is sold exclusively through 3M ESPE. Each gram of Clinpro Tooth Creme contains 0.95 mg of fluoride ion in a neutral pH base consisting of water, sorbitol, hydrated silica, glycerin, polyethylene-polypropylene glycol, flavor, polyethylene glycol, sodium lauryl sulfate, titanium dioxide, carboxymethyl cellulose, sodium saccharin and tri-calcium phosphate. This sodium lauryl sulfate prevents premature formation of calcium fluoride by its fluoride repelling surfactant action for remineralization even at deeper lesions. [20]

Previous studies have focused on casein phosphopeptide incorporated in sugar free gums[25,42], lozenges[5] and mouthwashes[36]. But there is lack of

information on effect of tri-calcium phosphate on white spot remineralisation in permanent and primary teeth.

The present in vitro study was conducted to evaluate the remineralization potential of Casein PhosphoPeptide - Amorphous Calcium Phosphate (Gc tooth mousse) and Tri-Calcium phosphate with 0.21%w/w sodium fluoride (Clinpro tooth crème) on artificial caries like lesions in primary and permanent teeth.

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REVIEW OF LITERATURE

Reynolds E.C, Cain C.J (1995)[33] investigated the Anticariogenicity of Calcium Phosphate Complexes of Tryptic Casein Phosphopeptides by the use of 144 specific pathogen free rats inoculated with streptococcus sorbinus. The animals were divided into 9 experimental groups based upon w/v % of CPP (0.1, 0.2, 0.5, and 1.0%), sodium phosphate and fluoride concentration or in combination. The CPP-ACP significantly reduced caries activity in a dose-response fashion, with 1.0% CPP-ACP producing 55% and 46% reductions in smooth surface and fissure caries activity respectively, being similar to that of 500 ppm F. The anitcariogenic effects with combination of CCP-ACP and fluoride were greater than when the agents were used alone.

Reynolds E.C (1997)[34] evaluated the remineralization of Enamel Subsurface Lesions by Casein Phosphopeptide-stabilized Calcium Phosphate Solutions. Enamel blocks from extracted third molars were used in this study were divided into 7 groups based upon % concentration of CPP (0.1, 0.5, 1.0) and pH (7.0, 7.5, 8.0, 8.5,9.0) of remineralising solution. After ten days of remineralisation period, enamel lesions were sectioned, subjected to microradiography and mineral content determined by microdensitometery. It was concluded that the remineralising capacity was greater for the solutions with higher levels of CPP-stabilized free calcium and phosphate ions with pH 7.0. The CPP, by stabilizing calcium and phosphate in solution, maintain high

concentration gradients of calcium and phosphate ions and ion pairs into subsurface lesions and thus affect high rates of enamel remineralization.

Shen P, Cai F (2001)[42] evaluated the ability of CCP-ACP in sugar free chewing gum to remineralise enamel subsurface lesions in a human in situ model. Thirty subjects wore removable palatal appliances with enamel half slabs containing demineralised lesions. Appliances were inserted immediately before gum chewing for 20 minutes and then retained for another 20 minutes. This was performed for four times per day for fourteen days. The results showed that the addition of CCP-ACP to either sorbitol or xylitol based chewing gum resulted in a dose-related increase in enamel remineralisation relative to control chewing gum.

Cai F, Shen P (2003)[5] determined the effect of CPP-ACP incorporation into a sugar-free lozenge (pressed mint tablet) on enamel remineralization in a human in-situ model. The study utilized a double-blind, randomized, cross-over design with four treatments: a lozenge containing 56.4mg CPP-ACP; a lozenge containing 18.8mg CPP-ACP; a lozenge not containing CPP-ACP and a no lozenge nil-treatment control. The enamel slabs were subjected to microradiography and computer-assisted densitometric image analysis to determine the level of remineralization. The incorporation of CPP-ACP into the lozenge significantly increased enamel subsurface lesion remineralization. Therefore they concluded that lozenges are a suitable vehicle for the delivery of CPP-ACP to promote its enamel remineralization potential.

Mazzaoui S.A, Burrow M (2003)[27] determined the effect of incorporating CPP-ACP into self cured glass ionomer cement. Cylinders of 4mm diameter×6mm long were made from the G.I.C's for the compressive strength test. Discs 6mm×2mm thick, using G.I.C with and without 1.56% CPP-ACP was allowed to set at 37degree centigrade and the calcium and phosphate concentrations were determined by atomic absorption spectrophotometry. It was concluded that the incorporation of CPP-ACP into the G.I.C significantly increased the compressive strength and significantly enhanced the release of calcium, phosphate and fluoride ions at neutral and acidic pH.

Reynolds E.C, Cai F (2003)[36] compared the ability of CPP-ACP with that of other forms of calcium, to be retained in supragingival plaque and remineralize enamel subsurface lesions in situ when delivered in a mouth rinse or a sugar free chewing gum in double blind trials. In the mouth rinse study only the CPP-ACP containing mouth rinse significantly increased plaque calcium and inorganic phosphate levels and the CPP were immunolocalised to the surfaces of bacterial cells as well as the intercellular matrix. In chewing gum studies, the gum containing the CPP-ACP produced the highest level of enamel remineralization independent of gum chewing frequency and duration.

Lijima Y, Cai F (2004)[25] studied the acid resistance of enamel subsurface lesions remineralized by a sugar- free chewing gum containing casein phosphopeptide-amorphous calcium phosphate. Ten healthy adults

were included in this in-situ study. Who used mid-palatal appliances with subsurface lesions of third molar. The study utilized a double blind, randomized, crossover design with two treatments a) sugar-free gum containing 18.8 mg of CPP-ACP and b) sugar-free gum not containing CPP-ACP as control. Subjects wore the appliance and chewed the gum for 20 min 4times per day for 14 days. After each treatment enamel half slabs were removed and half of each lesion challenged with acid for 8 or 16 hour. The level of remineralisation was determined by microradiography. The results showed that sugar-free gum containing containing CPP-ACP is superior to the control in remineralisation, with the remineralized mineral more resistant to subsequent acid challenge.

Tange Takashi, Yuko Sakurai (2004)[46] studied the effect of fluoride and xylitol on remineralization of enamel at the early stages of enamel caries on primary teeth. The samples were divided into four groups - control, 10% xylitol, 950ppm NaF, 10% xylitol + 950ppm NaF - and were analyzed using single thin section method and the pH cycling method In-vitro. Contact microradiographs were made to determine the mineral content. The remineralizing ratio were control - 8.9%, xylitol - 0.4%, NaF - 8.3% and NaF + Xylitol — *32.4%* respectively. Therefore it was assumed that the effect of fluoride and xylitol was additive and may be an effective caries preventive method.

Ramalingam L, Messer L (2005)[31] determined a minimal concentration of casein phosphopeptide stabilized amorphous calcium phosphate which when added to a sports drink would eliminate enamel erosion in vitro. Enamel specimens were immersed in sports drink powderade (PA; Coca-Cola, Sydney, Australia), powerade plus four concentrations of CPP-ACP (0.063%, 0.09%, 0.125%, 0.25%) or double deionised water. Enamel surface characteristics were examined under SEM. The results revealed that the pH of test solutions increased and the acidity decreased with increasing CPP-ACP concentrations.

Thaveesangpanich P, Itthagarun, (2005)[48] compared the effects of child formula toothpastes on enamel caries using two in vitro pH-cycling models. 40 Primary teeth were placed in demineralising solution for 96 hours to produce artificial carious lesions. They were cut into 100m thick sections and assigned to 6 groups. Sections in Groups A and D were exposed to a non-fluoridated toothpaste, those in Groups B and E to half-pea-sized (0.16g) and those in Groups C and F to pea-sized portions (0.32g) of a 500ppm F toothpaste. pH-cycling Model I (Groups A, B, C), without added fluoride, ran for 7 days, while Model II (Groups D, E, F), with 0.25ppm F, ran for 10 days. The two pH cycling models were for 7 and 10 days respectively. Lesions were evaluated using polarized light microscopy and microradiography. A pea-sized portion (0.32g) of 500ppm F toothpaste slowed down the demineralization progression better than a half-pea-sized portion.

Lennon M, Pfeffer M (2006)[24] compared the effect of casein/calcium phosphate cream, sodium fluoride and amine fluoride gel on enamel erosion. Sixty bovine enamel specimens were prepared and demineralised using 1% citric acid and were then distributed into five groups, followed by application of the three materials either alone or in combination and one group was not treated which was the control group. Evaluation of the erosive preventive effect was done using profilometer. It was concluded that the erosion – preventive effect of casein/calcium phosphate or 250ppm Sodium Fluoride either alone or in combination was less as compared to amine fluoride gel.

Arnold H Wolfang, Dorow Andreas (2006)[1] investigated the effect of four different toothpastes with differing fluoride compounds on enamel remineralisation using polarization light microscopy and quantitative energy dispersive X-ray analysis. Enamel surfaces of 90 premolars were demineralised in hydroxymethylcellulose solution at pH of 4.8. Teeth were divided into 6 groups and immersed in tooth paste slurry containing placebo paste (gp-1), remineralisation solution (gp-2), elemex anticaries (gp-3), elemex sensitive (gp- 4), blend-a-med complete (gp- 5), and colgate (gp-6). The teeth of each group was analyzed with polarized light microscopy and quantitative energy dispersive X-ray analysis. Results revealed an increase in calcium concentration and increased remineralisation of lesion body in the elmex anticaries group. It was concluded that amine fluoride compounds in toothpastes result in marked remineralisation of caries like lesions and the

quantifications of amount of mineral deposited is done with EDX elemental analysis.

L.J. Wang, Tang R (2006)[53] studied the Enamel Demineralization in Primary and Permanent Teeth. Twenty freshly extracted caries and filling-free human primary and permanent molars were used. The constant composition (CC) technique to investigate the acid-induced demineralization of these tissues at a relative undersaturation with respect to hydroxyapatite (HAP) of 0.902, pH = 4.5, and ionic strength = 0.15 mol L-1. The demineralization rates showed significant differences, primary enamel having the greater susceptibility to dissolution during an initial linear stage: 1.5 ± 0.5 x 10-10 mol mm-2 min-1 compared with 2.6 ± 0.5 x 10-11 mol mm-2 min-1 for permanent enamel.

Hegde N Mithra, Shishir Shetty (2007)[15] studied the Remineralization of enamel sub-surface lesion using casein phosphopeptide amorphous calcium phosphate (CPP-ACP) using SEM and a quantitative energy dispersive X-ray analysis (EDAX). 60 enamel specimens were prepared from extracted human molars. All specimens were evaluated for mineral (calcium and phosphorous) content (wt %) using EDAX. The specimens were placed in demineralizing solution for 48 hrs to produce artificial carious lesions. Mineral content was again measured using EDAX. The specimens were then randomly assigned to 3 study and 1 control group (incubated in artificial saliva after demineralization), in which each group

except the control was treated with remineralizing paste (10 % CPP-ACP paste) for 1, 5 and 10 days twice daily for 3 minutes, followed by incubation in artificial saliva at 37°C The control group received no treatment with remineralizing paste. After the remineralization treatment mineral content (wt %) of samples was measured using EDAX. The study groups showed an increase in the mineral content as compared to demineralized samples. No change was seen in the control group. 10 % CPP-ACP paste could significantly remineralize the artificial enamel sub-surface lesion in vitro and the remineralization potential was dose dependent.

Rana, Itthagarun (2007)[32] studied the Effects of dentifrices on artificial caries like lesions: an *in vitro* pH cycling study. Twenty seven sections from extracted third molar teeth were randomly divided equally into three groups. Sections in Group A were exposed to Vicco® (Vicco Laboratories, India), Group B to Perioe Children's Toothpaste® (LG, Korea) and Group C to Colgate Pokemon® (Colgate-Palmolive, Thailand). Polarised light microscopy and microradiography was used to evaluate lesion depth, before and after 10 days pH cycle. Mean lesion depths in Groups B and C reduced by 7% and 12% respectively, whereas lesion depth increased by 13% in Group A. It was concluded that the remineralising potential of Colgate Total® was higher than that of Perioe Cavity Care®.

Lynch, Ten Cate (2007)[26] studied the effect of lesion characteristics at baseline on subsequent de and remineralization behaviour. Thin enamel sections were prepared from extracted human premolars and bovine teeth divided into four groups. Artificial enamel lesions were prepared. One group was further demineralized, other group Lesions from both groups were remineralised. Remineralization was quantified microradiographically. Under demineralizing conditions there was marked decrease in further further mineral loss. The decrase in demineralization of lesions may be partially a result of increased intrinsic solubility through modified chemical composition. It was concluded that small lesions may be more vulnerable to demineralization. Large lesions may be more difficult remineralize.

Itthagarun, Rana (2007)[17] studied the Effects of child formula dentifrices on artificial caries like lesions using *in vitro* pH-cycling method. Twenty one sections from exfoliated primary teeth (molars, canines and maxillary incisors) were randomly divided equally into three groups. Sections in Group A were exposed to Perioe Children's Toothpaste® (LG, Korea), Group B to Colgate Pokemon® (Colgate-Palmolive, Thailand) and Group C to Vicco® (Vicco Laboratories, India). Polarised light microscopy and microradiography was used to evaluate lesion depth, before and after 7 days pH cycle. Mean lesion depths in Groups A and C increased by 11% and 14% respectively, while Group B demonstrated a lesion reduction of 3%. It was concluded that Colgate Pokemon® had the potential to remineralize the initial

carious lesions; Perioe Children's Toothpaste® dentifrice slowed down the progression of initial enamel carious lesions in primary teeth; however, it did not show an overall remineralising effect.

Oshiro M, Kanako Y (2007)[29] evaluated the effect of CPP-ACP paste on bovine teeth mineralization by observing the treated tooth surface using FE-SEM. The samples were divided into 4 groups one group was treated with 0.1M lactic acid for 10min before storing in artificial saliva, two additional groups of specimens were stored in 10 fold diluted CPP-ACP paste or placebo without CPP-ACP for 10min prior to storing in demineralising solution, other group was not treated. This procedure was carried out for 3,7,21 and 28 days and mineralization evaluation was done under FE-SEM. It was concluded that CPP-ACP treated specimens revealed lesser demineralization than control and negative controls. Therefore CPP-ACP paste was effective in preventing demineralization of enamel.

Kumar VLN, King NM (2008)[22] investigated the efficacy of CPP-ACP containing Tooth Mousse on the remineralization of enamel lesions and to compare its efficacy to that of a fluoride – containing tooth paste. 50 sound extracted third molars were placed in demineralising solution for 96 hours to produce artificial lesions. The samples and randomly assigned to five groups. Group A – Fluoridated tooth paste was used, Group- B: Non fluoridated tooth paste was used. Tooth Mousse containing CPP-ACP Was tested by three different means: as a tooth paste (Group C); as a topical coating (Group D)

and as a topical coating after treating the sections with same fluoridated paste (Group E). The lesion depth decreased significantly by 7 per cent in Group A, 10.1 per cent in Groups C and D, and 13.1 percent in Group E, while in Group B the lesion depth increased significantly by 23 per cent. It was concluded that CPP-ACP containing Tooth Mousse remineralized initial enamel lesions and it showed a higher remineralising potential when applied as a topical coating after the use of a fluoridated tooth paste.

Reynolds EC (2008)[37] determined the ability of CPP- ACP to increase the incorporation of fluoride into plaque and to promote enamel remineralization In-situ. In the study mouth rinses and dentifrices containing CPP- ACP and fluoride were used. The mouth rinses were used for 60 seconds, three times/day for 5 days and supragingival plaque was collected to analyze for Fluoride. The dentifrices were rinsed as water slurry for 60 seconds four times/day for 14 days in an in-situ model. The addition of 2% CPP-ACP to the 450 ppm F mouthrinse significantly increased the incorporation of fluoride into plaque. The dentifrice containing 2% CPP-ACP produced a level of remineralization similar to that achieved with a dentifrice containing 2800 ppm. The dentifrice containing 2% CPP-ACP plus 1100 ppm F was superior to all other formulations.

Karlinsey R (2009)[19] conducted an *in vitro* pH cycling experiment to assess the fluoridating and remineralizing efficiency of Clinpro Tooth Crème and GC MI Paste Plus. In this experiment, specimens were subjected to 20

days of pH cycling. Enamel chips were prepared from bovine incisors. The tooth surfaces were ground flat and then polished. The indigenous fluoride level of each chip was determined. The chips were demineralised using a solution of 0.1M lactic acid and 0.2% carbopol. Following demineralization, the chips were treated for 30 minutes by soaking in a slurry of fluoride preparation and water. The fluoride preparations consisted of the following: • Fluoride-free deionized water • GC MI Paste Plus™ (900 ppm F-) • Clinpro Tooth Crème (950 ppm F-) Following treatment, chips were re-analyzed using the same technique used to determine indigenous fluoride level. The fluoride level after treatment was compared to fluoride level before treatment to determine fluoride uptake. Results showed Clinpro Tooth Creme exhibited statistically significantly greater fluoride uptake than GC MI Paste Plus. This suggests that the anticaries potential of Clinpro Tooth Creme is greater than that of GC MI Paste Plus.

Fan Y, Sun Z (2009)[10] examined the effect of fluoride ion concentration on the morphology of calcium phosphate crystals grown on acid etched enamel as a model for tooth enamel erosion. Human third molar Samples were immersed in calcification solution for 16 hrs and changes in crystal morphology were monitored by field emission scanning electron microscopy. It was concluded that fluoride had dose dependent effect on crystal morphology, introducing as little as 1mg/l fluoride was effective in altering the crystal nanostructure.

Walker GD, Cai F (2009)[51] studied the Consumption of milk with added casein phosphopeptide amorphous calcium phosphate and its capacities to remineralizes enamel subsurface lesions in situ. Ten subjects drank 100 mL of bovine milk containing no added CPP-ACP (control milk), 0.2% (w / v) CPP-ACP or 0.3% (w / v) CPP-ACP, for 30 seconds once daily for 15 days, whilst wearing removable appliances with attached slabs of enamel containing subsurface enamel lesions. After each treatment and a one-week washout period, subjects crossed over to another treatment and this was repeated until they had consumed each of the three milk products. At the completion of each treatment the enamel slabs were removed and remineralization was determined using microradiography. The remineralizing effect of CPP-ACP in milk was dose-dependent with milk containing 0.2% CPP-ACP and 0.3% CPP-ACP producing an increase in mineral content of 81% and 164%, respectively, relative to the control milk.

Karlinsey L, Mackey A (2010)[20] studied the Remineralization potential of 5,000 ppm fluoride dentifrices evaluated in a pH cycling model. Twelve Bovine enamel Specimens were used, then measured for baseline Vickers microhardness and stratified into the following groups: Group A: Tom's of Maine fluoride-free dentifrice (negative control); Group B: Colgate PreviDent® Booster 5000 (5000 ppm fluoride) and Group C: 3M Clinpro® 5000 (5000 ppm fluoride). The groups were then cycled for 10 days in a pH cycling model consisting of four one-minute treatment periods (diluted 1:3

with distilled water) and one four-hour acid challenge (lactic acid-PAA, pH = 5.0) per day. Between these events, specimens were immersed in artificial saliva (pH = 7.0). After 10 days of cycling, the specimens were evaluated for Vickers surface microhardness, mineral loss and lesion depth using microindentation, transverse microradiography and polarized light microscopy. Statistical analysis showed Clinpro® 5000 conferring superior surface and subsurface remineralization potential relative to both PreviDent® Booster 5000 and Tom's of Maine fluoride-free paste. Due to this superiority, these results suggest the combination of 5,000 ppm fluoride plus the tricalcium phosphate system may provide significant anticaries benefits relative to fluoride-only and fluoride-free dentifrices.

Badr Y Sherine, Ibrahim (2010)[3] studied the Protective effect of three different fluoride pre treatments on artificially induced dental erosion in primary and permanent teeth. Sixty extracted human primary molars (n = 30) and young permanent premolars (n = 30) were used in this study. The coronal portion of each tooth was sectioned mesio-distally. Specimens were prepared by embedding the crown sections in acrylic resin blocks leaving the enamel surfaces exposed. Specimens were ground, polished and randomly assigned to one of three groups each of 10 according to the protective agent used: APF gel (1.23% F), NaF varnish (0.1%F), and CPP-ACPF paste (0.2%F). Half of the exposed enamel surface was protected with adhesive tape during the treatment of the remaining surface according to their group. Six daily demineralization–

remineralization cycles of 5 minutes of immersion in a cola drink (pH 2.3) and 30 minutes in artificial saliva were conducted for 14 days. Surface Vickers Micro-hardness readings were recorded at baseline and 14 days later for both halves. Percentage surface microhardness reduction (%SMHR) was then calculated. All of the tested fluoride treatments were able to reduce erosive enamel loss in both primary and permanent groups. In primary teeth only APF gel showed significantly higher anti-erosive effect than both fluoride varnish and CPP-ACPF paste. In permanent teeth both CPP-ACPF paste and APF gel showed significantly higher protective anti-erosive effect than fluoride varnish.

Rirattanapong P, Smutkeere A (2010)[39] studied the effect of fluoride dentrifice on remineralisation of demineralised primary enamel. One hundred twenty primary incisors were partly covered with a nail varnish, leaving a 1x1 mm window, then placed in demineralising solution for 96 hours to produce artificial carious lesions 60-100 μm in depth. They were assigned to 8 groups (A to H; *n*=15). Groups A-D were exposed to a half pea-sized portion of dentifrice (0.16 g) and groups E-H were exposed to a pea-sized portion of dentifrice (0.32 g), both groups with fluoride concentrations of 0, 250, 500 and 1,000 ppm. The pH-cycling method was carried out for 7 days, then the teeth were cut through the lesions and examined under a polarized light microscope, photographs were taken and analyzed. Lesion depth was measured using a computerized method using the Image-Pro® Plus Program. The results were

analyzed using the one way ANOVA and LSD tests. The mean lesion depth in the 2 non-fluoridated control groups (A and E) was significantly deeper than in the fluoridated groups. There were no differences found between the half peasized and pea-sized dentifrice.

MATERIALS AND METHODS

The present study was conducted by Department of Pedodontics and Preventive Dentistry, Ragas Dental College and Hospital, Chennai in association with department of mechanical engineering, College of Engineering, Anna University to evaluate the potential of two remineralizing agents with different Calcium systems having anti-caries effect, in primary and permanent teeth.

Twenty human primary teeth divided randomly into two groups of ten each and twenty human permanent teeth randomly into two groups of ten each were selected for the study according to the agents used.

INCLUSION CRITERIA

- Teeth without carious lesion.
- Sound teeth with all surfaces intact.

EXCLUSION CRITERIA

- Teeth with cracks
- Teeth with hypoplasia
- Teeth with caries on any surface
- Teeth with white spot lesions

MATERIALS

- Twenty human primary teeth
- Twenty human permanent teeth.
- Applicator brush
- Plastic containers
- Acid resistant nail varnish (Revlon)
- Demineralizing solution
- Remineralizing solution
- Remineralizing paste with TCP O.21% w/w sodiumfluoride (CLINPRO TOOTH CREME)
- Remineralizing paste with CPP-ACP (GC Tooth Mousse)
- Scanning Electron Microscope (SEM)
- Energy Dispersive X Ray Machine (EDAX)

METHODOLOGY

Twenty intact human primary and twenty human permanent teeth were collected cleaned with curettes and ultrasonic tips to remove any calculus, soft tissue debris and then stored in 0.5% physiologic saline until use. The teeth with cracks, hypoplasia, white spot lesions, and caries were excluded from the study.

Twenty human primary teeth divided randomly into two groups of ten each and twenty human permanent teeth randomly divided into two groups of ten each were selected for the study according to the agents used.

GROUP I - CPP-ACP (GC Tooth mousse) in permanent teeth

GROUP II- TCP with 0.21%w/w sodium fluoride (CLINPRO) in Permanent teeth

GROUP III- CPP-ACP (GC Tooth mousse) in Primary teeth

GROUP IV- TCP with 0.21%w/w sodium fluoride (CLINPRO) in Primary teeth

LESION FORMATION

All the teeth were coated with an acid resistant nail varnish (Revlon, USA) leaving two narrow rectangular windows (occlusal 1/3 and gingival 1/3) of approximately 1mm size, on the intact buccal surface.

The teeth were then immersed in demineralizing solution (10ml per tooth) for 96hrs. Visual examination was performed to confirm the presence of artificial caries lesions.

After the de-mineralization was observed, *gingival window* of each tooth is closed with nail varnish which serves as a de-mineralisation control, leaving the *occlusal window* for topical application of the remineralization agent.

PREPARATION OF THE DEMINERALISING AND REMINERALISING SOLUTION:

The buffered ***demineralizing and remineralizing solution*** was made of analytical-grade chemicals in department of biochemistry, Ragas Dental College.

The composition of ***demineralizing solution*** contained 2.2Mm $CaCl_2$, 2.2 Mm KH_2PO_4, 0.05M acetic acid at the ph adjusted to 4.4.

The composition of ***remineralizing solution***, was 1.5 mM $CaCl_2$, 0.9 mM NaH_2PO4, 0.15 M KCL had a pH of 7.0. This solution approximates to the super saturation of apatitic minerals found in saliva and was similar to that utilized by **ten cate and duijsters[47] and VLN Kumar, NM king[22].**

REMINERALIZING AGENT APLICATION:

1. **Group-I**: CPP-ACP paste (GC tooth mousse) was applied topically with a microbrush twice daily for three minutes on the exposed occlusal window of each tooth followed by immersion in remineralizing solution for ten days. Remineralizing solution was changed everyday.
2. **Group-II:** TCP Paste with 0.21%w/w sodium fluoride (CLINPRO TOOTH CRÈME) was applied topically with a microbrush twice daily for three minutes on the exposed occlusal window of each tooth followed by immersion in remineralizing solution for ten days. Remineralizing solution was changed everyday.
3. **Group-III:** CPP-ACP Paste (GC tooth mousse) was applied topically with a microbrush twice daily for three minutes on the exposed occlusal window of each tooth followed by immersion in remineralizing solution for seven days. Remineralizing solution was changed everyday.

4. **Group-IV:** TCP Paste with 0.21%w/w sodium fluoride (CLINPRO TOOTH CRÈME) was applied topically with a microbrush twice daily for three minutes on the exposed occlusal window of each tooth followed by immersion in remineralizing solution for seven days. Remineralizing solution was changed everyday.

EVALUATION TECHNIQUES:

Remineralization potential was evaluated qualitatively (SEM) and quantitatively (EDAX) in the college of engineering, Anna university campus.[15] Before evaluation nail varnish was completely removed from the tooth with the help of Acetone[22]. The surface above the occlusal window shows normal enamel, the gingival window acts as a demineralization control and occlusal window acts as remineralization control.

Qualitative evaluation:

SCANNING ELECTRON MICROSCOPE:

Enamel surface of the specimens were qualitatively evaluated using scanning electron microscopy. Any changes that occurred in the lesions during the experimental period could be detected from the images (gold coating was done according to the specification for SEM examination), which were taken at the same magnification before and after the experiment.

Three images with best clarity per specimen were included:

1. One was to see the normal enamel surface above the occlusal 1/3 window.
2. Second image to see surface change of each specimen after demineralization. (Gingival 1/3 window)
3. Third image to see surface change of each specimen after remineralization. (Occlusal 1/3 window)

Quantitative evaluation:

EDAX ANALYSIS:

EDAX Analysis stands for energy dispersive X-ray analysis. It is sometimes also referred to as EDS or EDAX analysis. It is a technique used for identifying the elemental composition in the samples. The EDAX system works as an integrated feature of scanning electron microscope. The mineral content can be digitally interpreted wherein the output is given in numerical values for each mineral component. The EDAX analysis was performed in three sites as mentioned above.

FIGURE 1 : HUMAN PERMANENT TEETH

FIGURE 2 : HUMAN PRIMARY TEETH

FIGURE 3A: REMINERALIZING AGENTS AND NAIL VARNISH

FIGURE 3B : DEMINERALIZING AND REMINERALIZING SOLUTION

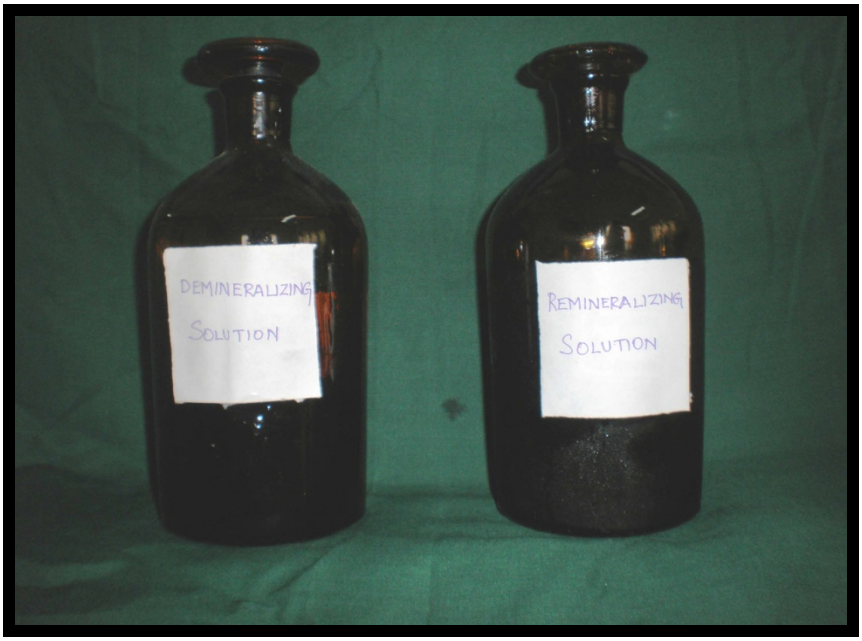


FIGURE 4A: SAMPLES OF GROUP – I CPP – ACP (PERMANENT)

FIGURE4B : SAMPLES OF GROUP – II TCP (PERMANENT)

FIGURE 4C : SAMPLES OF GROUP – III CPP – ACP (PRIMARY)

FIGURE 4D: SAMPLES OF GROUP – IV TCP (PRIMARY)

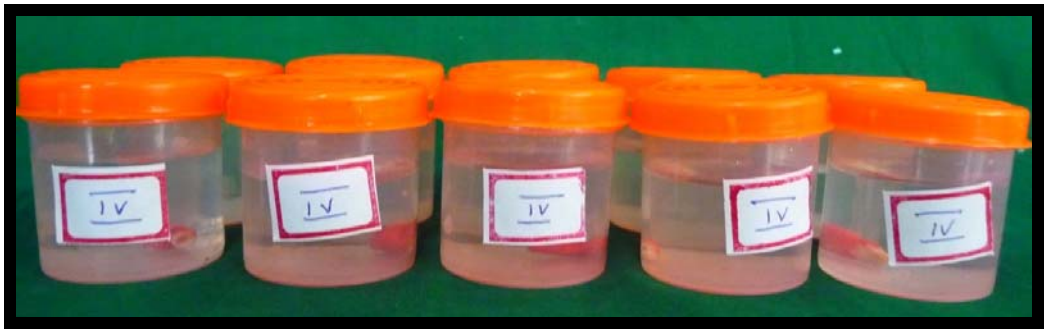


FIGURE 5 : TOPICAL APPLICATION OF REMINERALIZING PASTE

FIGURE 6 : SEM – EDAX MACHINE

RESULTS

Energy dispersive X-ray analysis was used to determine Calcium and Phosphorus content in weight % at baseline, demineralized and remineralized enamel in each group. The calcium and phosphorus content was interpreted as Ca/P ratios in each group [Table 1], [Table 2], [Table 3], [Table 4].

The results of the present study were subjected to statistical analysis**.** The SPSS software was used to calculate One-Way ANOVA followed by *post hoc* Tukey HSD.

The significant differences between various Ca/P ratios of baseline, demnieralised and remineralized specimens between the groups were estimated.

TABLE - 1

GROUP-I: CPP-ACP PERMANENT TEETH (GC TOOTH MOUSSE)

CALCIUM-PHOSPHOROUS WEIGHT % RATIO

Sample	**Baseline**	**Demineralised**	**Remineralised**
1	1.84	1.58	1.74
2	1.86	1.59	1.77
3	1.89	1.72	1.77
4	1.91	1.62	1.76
5	1.94	1.63	1.79
6	1.97	1.65	1.73
7	2.01	1.66	1.71
8	1.86	1.68	1.79
9	1.96	1.70	1.76
10	2.01	1.71	1.77
MEAN	**1.925**	**1.654**	**1.759**

TABLE I represents Ca/P ratios of samples from Group I (CPP-ACP GC TOOTH MOUSSE – PERMANENT). The Ca/P ratio ranged between 2.01 – 1.84 at baseline 1.72 – 1.58 on demineralization and 1.79 – 1.71 on remineralisation. The mean baseline value was 1.925 from which it was demineralised to mean value of 1.654 and was remineralised to mean value of 1.759

TABLE - 2

GROUP-II: TCP PERMANENT TEETH (CLINPRO)

CALCIUM-PHOSPHOROUS WEIGHT % RATIO

Sample	**Baseline**	**Demineralised**	**Remineralised**
1	1.82	1.57	1.70
2	1.85	1.59	1.73
3	1.88	1.60	1.68
4	1.83	1.61	1.70
5	1.93	1.63	1.73
6	1.96	1.64	1.73
7	2.03	1.66	1.76
8	2.01	1.67	1.74
9	1.93	1.69	1.73
10	2.07	1.71	1.75
MEAN	**1.931**	**1.637**	**1.725**

TABLE II represents the Ca/P ratios of the samples from GROUP II (TCP CLINPRO – PERMANENT). The Ca/P ratio ranged between 2.07 – 1.82 at baseline, 1.71 – 1.57 on demineralization and 1.76 – 1.68 on remineralisation. The mean baseline value was 1.931 from which it was demineralised to mean value of 1.637 and was remineralised to mean value of 1.725.

TABLE - 3

GROUP-III: CPP-ACP PRIMARY TEETH (GC TOOTH MOUSSE)

CALCIUM-PHOSPHOROUS WEIGHT % RATIO

Sample	**Baseline**	**Demineralised**	**Remineralised**
1	1.88	1.44	1.67
2	1.81	1.42	1.65
3	1.84	1.43	1.62
4	1.90	1.50	1.69
5	1.82	1.50	1.71
6	1.80	1.51	1.70
7	1.87	1.48	1.67
8	1.81	1.47	1.71
9	1.85	1.46	1.68
10	1.77	1.41	1.66
MEAN	**1.835**	**1.462**	**1.676**

TABLE III represnts Ca/P ratios of the samples from GROUP III (CPP-ACP GC TOOTH MOUSSE – PRIMARY). The Ca/P ratio ranged between 1.90 – 1.77 at baseline, 1.51 – 1.41 on demineralization and 1.71 – 1.62 on remineralisation. The mean baseline value was 1.835 from which it was demineralised to mean value of 1.462 and was remineralised to mean value of 1.676.

TABLE - 4

GROUP-IV: TCP PRIMARY TEETH (CLINPRO)

CALCIUM-PHOSPHOROUS WEIGHT % RATIO

Sample	**Baseline**	**Demineralised**	**Remineralised**
1	1.81	1.41	1.61
2	1.86	1.46	1.60
3	1.83	1.43	1.62
4	1.87	1.47	1.66
5	1.89	1.48	1.63
6	1.82	1.42	1.60
7	1.87	1.48	1.64
8	1.88	1.49	1.68
9	1.84	1.44	1.63
10	1.85	1.45	1.56
MEAN	**1.852**	**1.453**	**1.623**

TABLE IV represents Ca/P ratios the samples from GROUP IV (TCP CLINPRO – PRIMAY). The Ca/P ratio ranged between 1.89 – 1.81 at baseline, 1.49 – 1.41on demineralization and 1.68 – 1.56 on remineralisation. The mean baseline value was 1.852 from which it was demineralised to mean value of 1.453 and was remineralised to mean value of 1.623.

TABLE - 5

REMINERALIZATION POTENTIAL OF THE TWO PASTES AMONG THE STUDY GROUPS

	Stages						Remineralisation change
	Baseline		Demineralisation		Remineralisation		
	Mean	SD	Mean	SD	Mean	SD	
Group I	1.925	.062	1.654	.049	1.759	.026	0.105 ± 0.0512
Group II	1.931	.087	1.637	.045	1.725	.025	0.088± 0.0330
Group III	1.835	.040	1.462	.036	1.676	.028	0.214± 0.0232
Group IV	1.852	.027	1.453	.028	1.623	.034	0.170± 0.0290

TABLE V shows the mean and the standard deviation of the Ca/P values for each group at baseline, on demineraliastion and remineralisation.

In **Group I** and **II** (permanent teeth) mean remineralization change is 0.105 ± 0.0512 and 0.088± 0.0330 respectively.

In **Group III** and **IV** (primary teeth) mean remineralization change is 0.214± 0.0232 and 0.170± 0.0290respectively.

TABLE - 6

COMPARISON OF REMINERALIZATION POTENTIAL OF TWO PASTES AMONG STUDY GROUPS

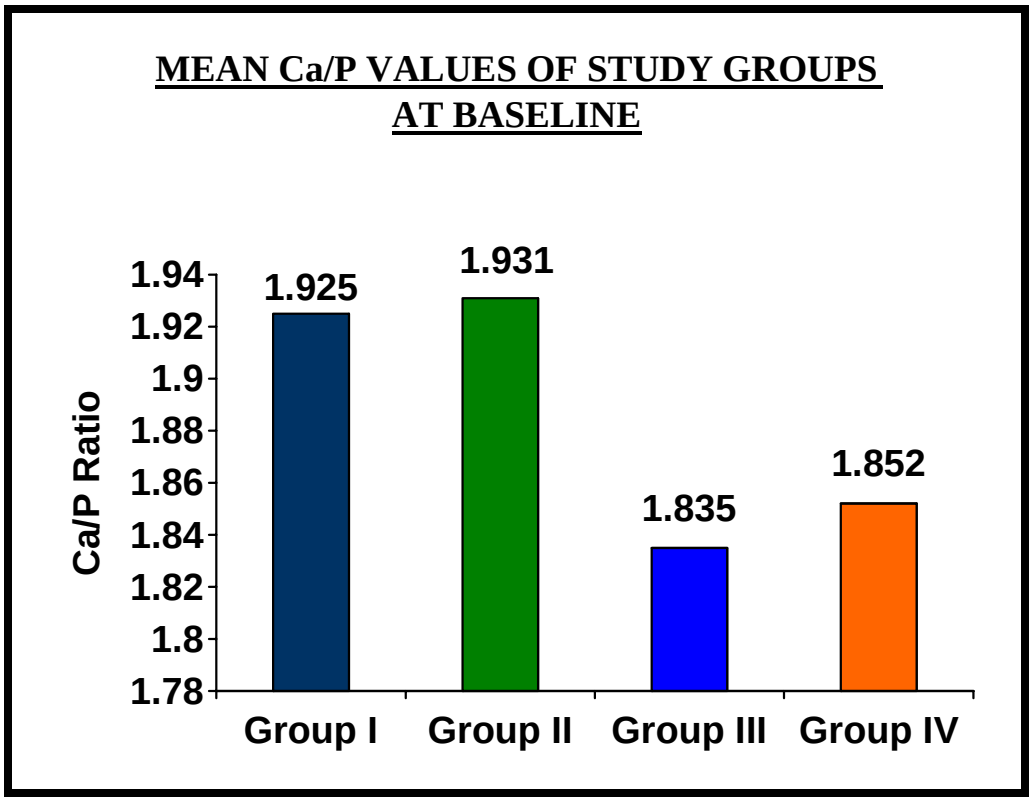
TEETH / **AGENT**	**PERMANENT (MEAN=S.D)**	**PRIMARY (MEAN+S.D)**
CPP-ACP (G.C TOOTH MOUSSE)	0.105	0.214
TCP (CLINPRO)	0.088	0.170
	I (Vs) II (P = 0.389)	II (Vs) IV **(P = 0.001)**

TABLE VI shows the comparison of the *remineralising pastes CPP-ACP* (G.CTOOTH MOUSSE) AND TCP (CLINPRO).

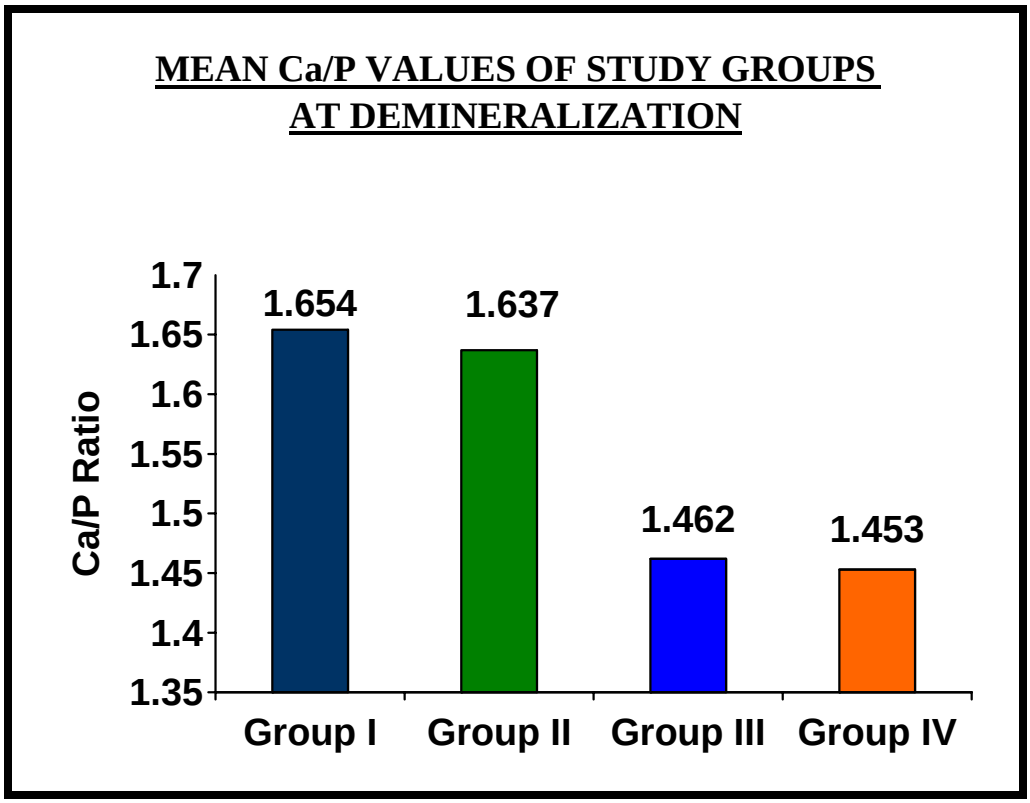
When the mean Ca/P values were compared in between the REMINERALISING PASTES (**GROUP I** and **GROUP II**) in PERMANENT TEETH there was higher remineralisation in case of **GROUP I** (CPP-ACP G.C TOOTH MOUSSE) but this change was not statistically significant. (p = 0.389)

When the mean Ca/P values were compared in between the REMINERALISING PASTES (**GROUP III** and **GROUP IV**) in PRIMARY TEETH there was higher remineralisation in case of **GROUP III** (CPP-ACP G.C TOOTH MOUSSE) this change was statistically significant.(p = 0.001)

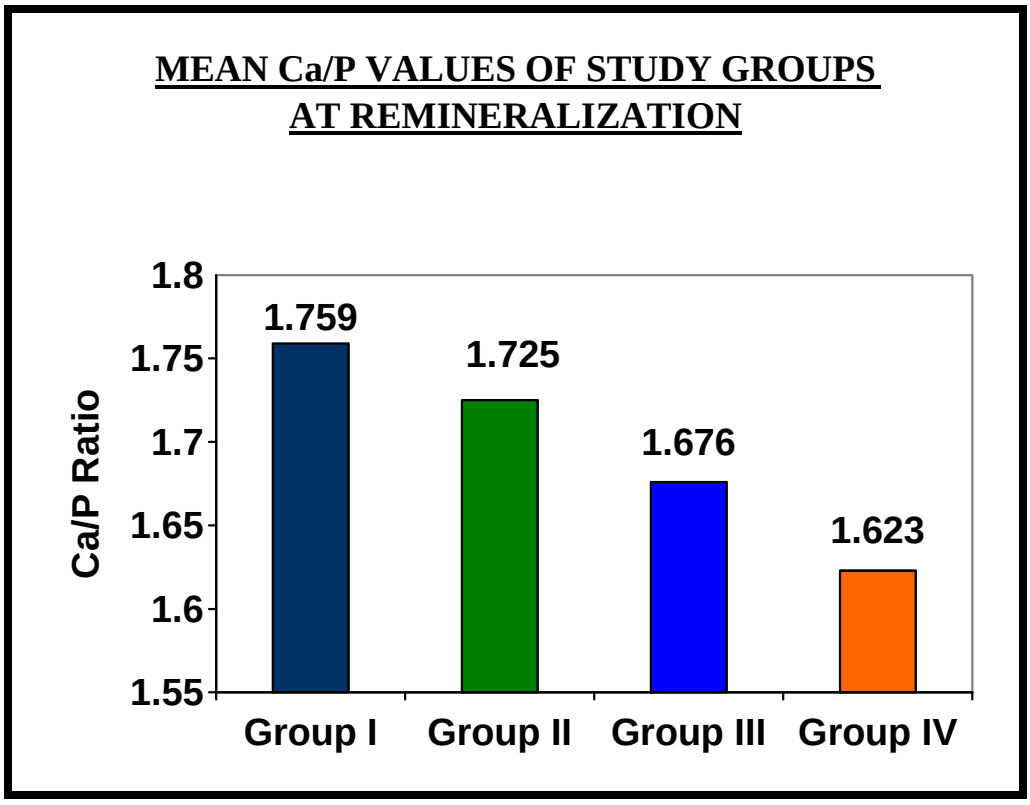
GRAPH – 1


MEAN Ca/P VALUES OF STUDY GROUPS AT BASELINE
Ca/P Ratio
1.94
1.92
1.9
1.88
1.86
1.84
1.82
1.8
1.78
1.925
1.931
1.835
1.852
Group I
Group II
Group III
Group IV

GRAPH – 2


MEAN Ca/P VALUES OF STUDY GROUPS AT DEMINERALIZATION
Ca/P Ratio
1.7
1.65
1.6
1.55
1.5
1.45
1.4
1.35
1.654
1.637
1.462
1.453
Group I
Group II
Group III
Group IV

GRAPH – 3


MEAN Ca/P VALUES OF STUDY GROUPS AT REMINERALIZATION
Ca/P Ratio
1.8
1.75
1.7
1.65
1.6
1.55
1.759
1.725
1.676
1.623
Group I
Group II
Group III
Group IV

GRAPH – 4

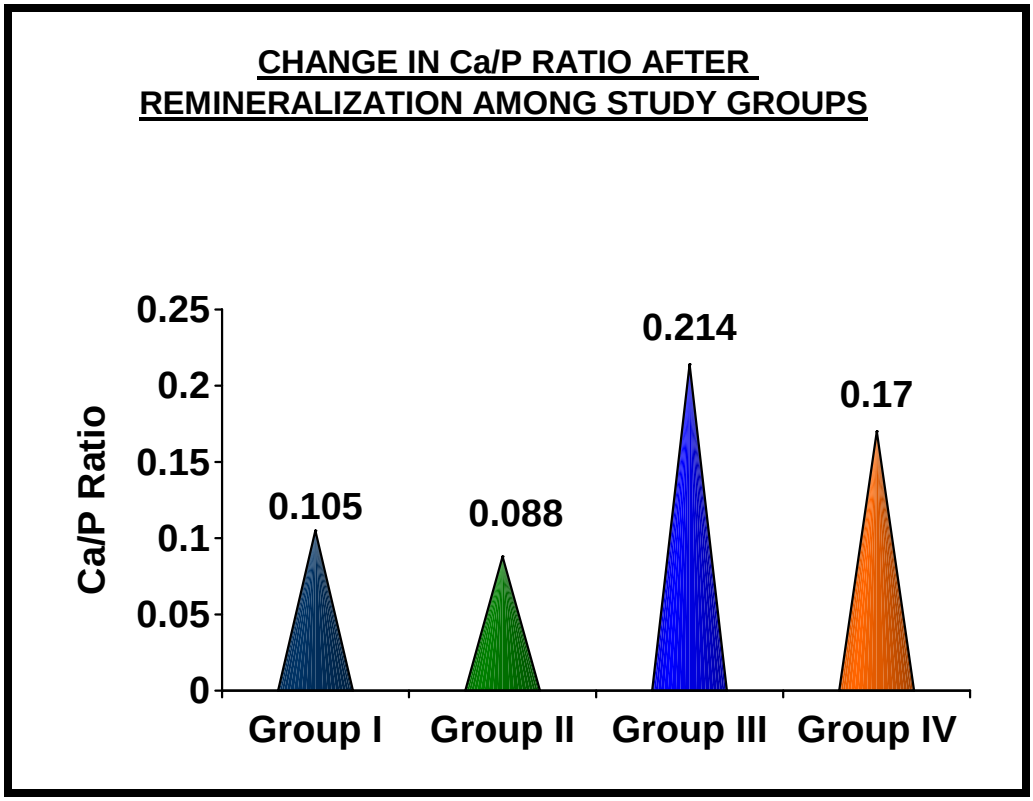
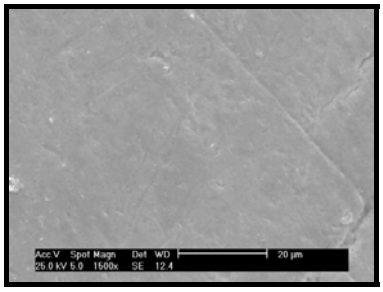

CHANGE IN Ca/P RATIO AFTER REMINERALIZATION AMONG STUDY GROUPS
Ca/P Ratio
0.25
0.2
0.15
0.1
0.05
0
0.105
0.088
0.214
0.17
Group I
Group II
Group III
Group IV

FIGURE : 7A

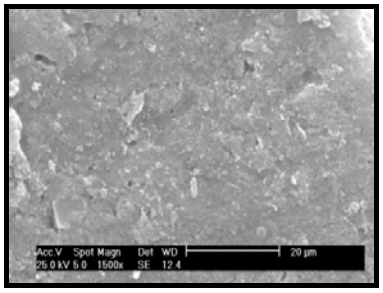
SCANNING ELECTRON MICROSCOPIC IMAGES

GROUP – I

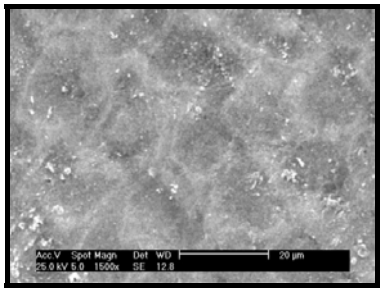
CPP – ACP (PERMANENT TEETH)



BASELINE



DEMINERALIZATION



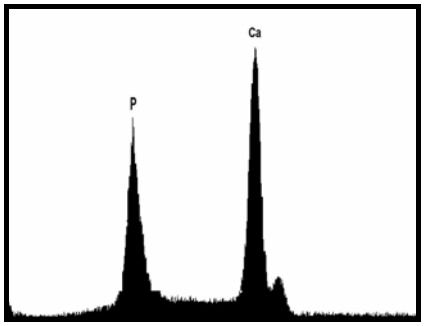
REMINERALIZATION

FIGURE : 7B

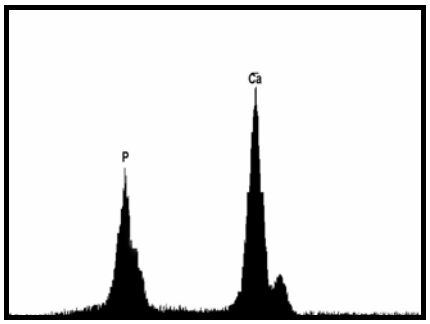
GRAPHIC INTERPRETATION OF ENERGY DISPERSIVE X-RAY ANALYSIS

GROUP – I

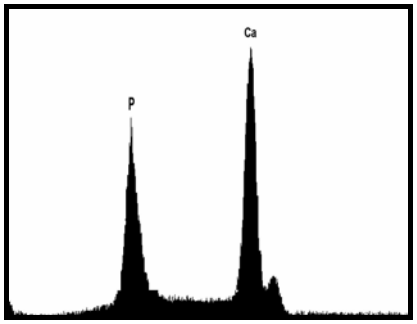
CPP – ACP (PERMANENT TEETH)



BASELINE



DEMINERALIZATION



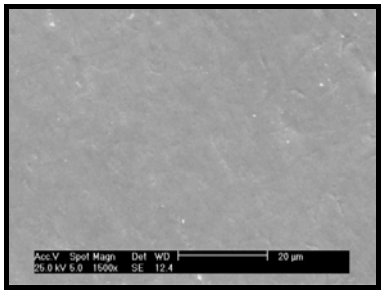
REMINERALIZATION

FIGURE : 8A

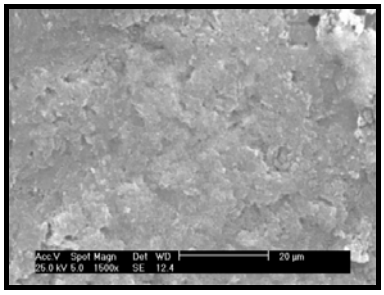
SCANNING ELECTRON MICROSCOPIC IMAGES

GROUP – II

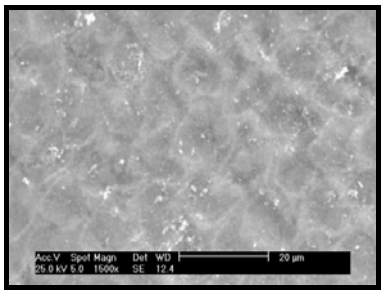
TCP (PERMANENT TEETH)



BASELINE



DEMINERALIZATION



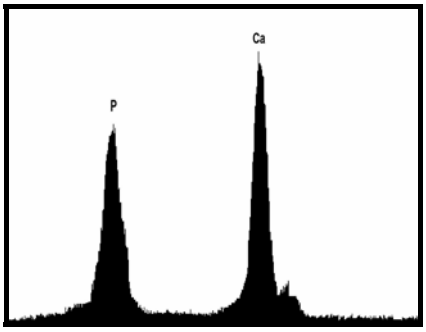
REMINERALIZATION

FIGURE : 8B

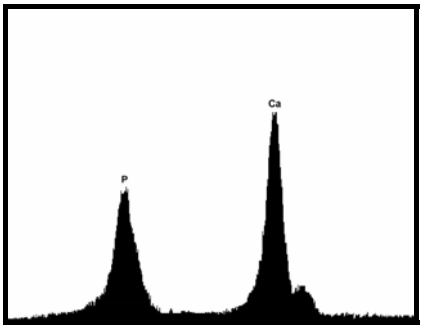
GRAPHIC INTERPRETATION OF ENERGY DISPERSIVE X-RAY ANALYSIS

GROUP – II

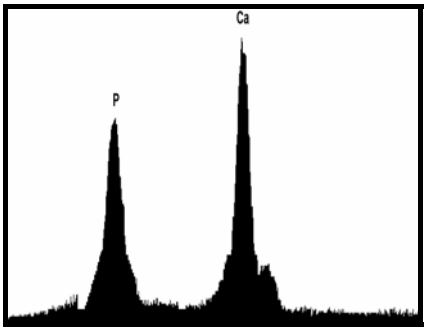
TCP (PERMANENT TEETH)



BASELINE



DEMINERALIZATION



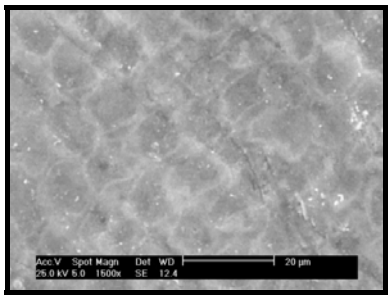
REMINERALIZATION

FIGURE : 9A

SCANNING ELECTRON MICROSCOPIC IMAGES

GROUP – III

CPP – ACP (PRIMARY TEETH)



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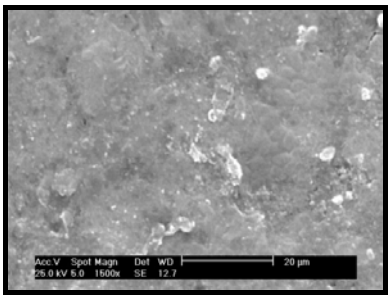

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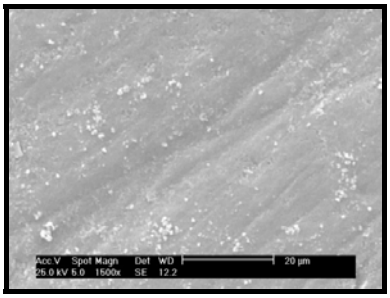


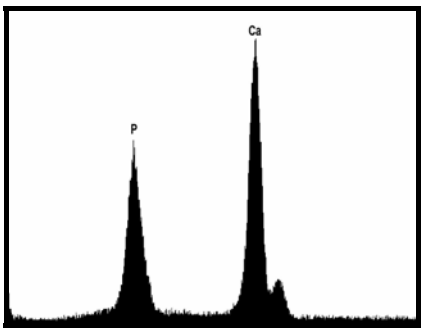
REMINERALIZATION

FIGURE : 9B

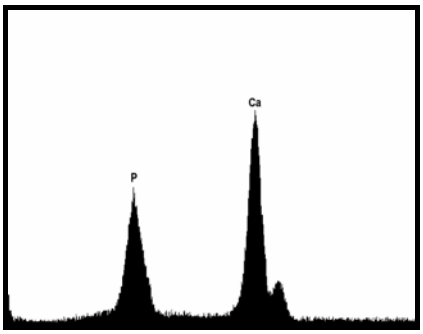
GRAPHIC INTERPRETATION OF ENERGY DISPERSIVE X-RAY ANALYSIS

GROUP – III

CPP – ACP (PRIMARY TEETH)



BASELINE



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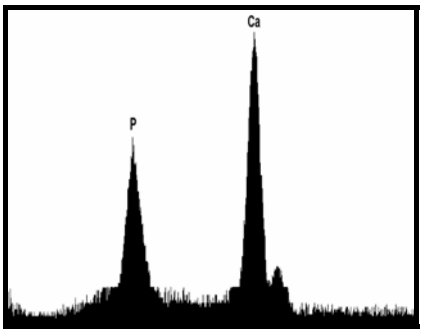

REMINERALIZATION

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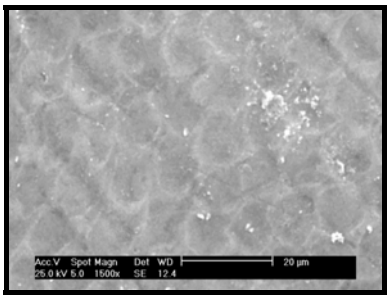
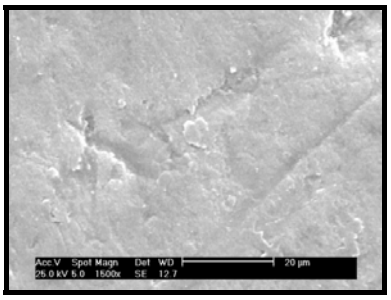
FIGURE : 10A

SCANNING ELECTRON MICROSCOPIC ~~ANALYSIS~~ IMAGES ~~PRIMARY~~

GROUP – IV

TCP (PRIMARY)





BASELINE **DEMINERALIZATION**

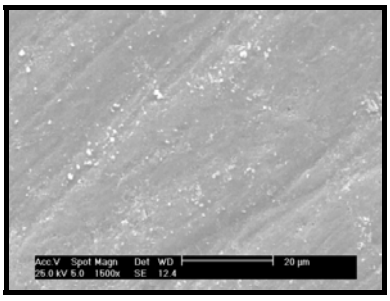


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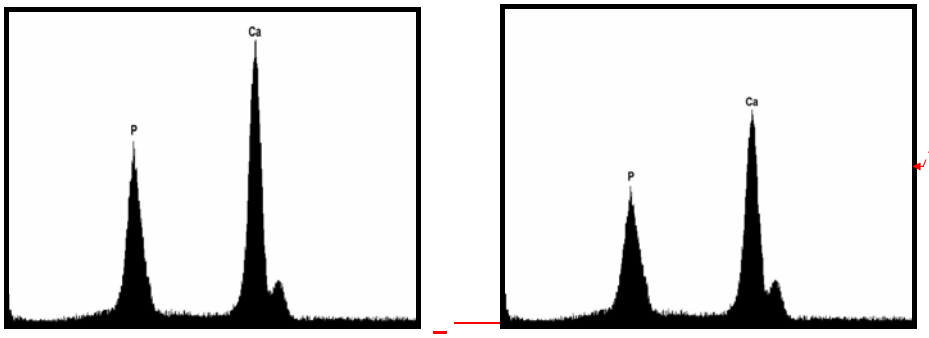
FIGURE : 10B

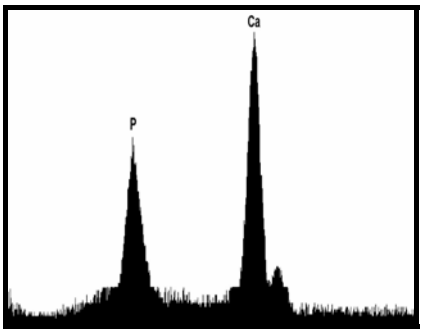
GRAPHIC INTERPRETATION OF ENERGY DISPERSIVE X-RAY ANALYSIS

GROUP – IV

TCP (PRIMARY)



BASELINE **DEMINERALIZATION**



DEMINERALIZATION

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DISCUSSION

Dental caries remains a major public health problem in most industrialized countries, affecting up to 90% of schoolchildren.[17] It is also the most prevalent oral disease. Caries is an active and dynamic biological process. In its early phase, demineralization occurs in the enamel and can be reversed through remineralization under favorable conditions. Demineralization results when cariogenic bacteria metabolize fermentable carbohydrates to produce acids[12]. The acids attack the surface of the tooth, causing demineralization of the hydroxyapatite crystals. Calcium, phosphate, and hydroxyl ions are lost from the hydroxyapatite crystals, thereby weakening the structure. The acids also diffuse into the fluid-filled spaces within the mineral crystals, causing subsurface demineralization and dissolution of the crystal structure[8].

Demineralization is first visible as a "white spot lesion" on the surface of the tooth enamel. Left untreated, this process can continue, eventually leading to cavitation, the early white spot lesions can be intervened by application of preventive strategies like fluoride application[1,14,37], use of xylitol chewing gums[46], Glass ionomer cement[27,49] and application of remineralizing materials like casein phosphopeptides amorphous calcium phosphate(GC TOOTH MOUSSE)[1,7,34], and several other calcium compounds in dentifrices and a very recently introduced remineralization material

Tri calcium phosphate with 0.21% w/w Sodium Fluoride (CLINPRO TOOTH CRÈME)[20].

Requirements of an ideal remineralization material include: diffuses into the subsurface, or delivers calcium and phosphate into the subsurface, does not deliver an excess of calcium, does not favour calculus formation, works at an acidic pH, Works in xerostomic patients and Boosts the remineralizing properties of saliva[52].

GC Tooth mousse is water based, lactose free cream containing casein phosphopeptide and amorphous calcium phosphate (CPP-ACP). When CPP-ACP is applied in the oral environment, it will bind to biofilms, plaque, bacteria, hydroxyapatite and soft tissue localizing bio-available calcium and phosphate.

CPP-ACP (GC tooth mousse) technology was developed by Eric ***Reynolds and coworkers (2003)*** at the University of Melbourne, and has since been incorporated into chewing gums (such as Recaldent gum™ and Trident White™) and tooth crèmes (GC Tooth Mousse™ and MI Paste™). A formulation with incorporated fluoride to a level of 900 ppm (GC Tooth Mousse Plus™, MI Paste Plus™) is also available. This protein nanotechnology combines specific phosphoproteins from bovine milk while forming nanoparticles of amorphous calcium phosphate (ACP) [36]. The precise ratio is 144 calcium ions plus 96 phosphate ions and 6 peptides of CPP. The casein phosphopeptides (CPP) are produced from a tryptic digest of the milk

protein casein, then aggregated with calcium phosphate and purified by ultrafiltration. Under alkaline conditions the calcium phosphate is present as an alkaline amorphous phase complexed by the CPP. The nano-complexes form over a pH range from 5.0 to 9.0. Under neutral and alkaline conditions, the casein phosphopeptides stabilize calcium and phosphate ions, forming metastable solutions that are supersaturated with respect to the basic calcium phosphate phases. The amount of calcium and phosphate bound by CPP increases as pH rises, reaching the point where the CPP have bound their equivalent weights of calcium and phosphate, so that spontaneous precipitation of calcium phosphate does not occur.

CPP-ACP works effectively as a remineralizing agent at acidic pH levels (down to 4.0) as well as in the neutral and alkaline range. The extent of remineralization seen with CPP-ACP does not significantly correlate with levels of CPP-bound ACP or the degrees of saturation for hydroxyapatite, octacalcium phosphate or ACP. Rather, there is a strong correlation between remineralization and the concentration of the neutral ion pair $CaHPO_4$. By stabilizing calcium phosphate in solution, the CPP maintain high-concentration gradients of calcium and phosphate ions and ion pairs into subsurface lesions, an effect which explains the high rates of enamel subsurface remineralization which can be achieved when these products are used in solutions, gums, lozenges and crèmes. CPP-ACP incorporated into chewing gum, lozenges and mouthrinses has been shown to re-mineralize

enamel subsurface lesions and its efficacy is attributed to its similarity to other proteins which stabilize calcium and phosphate in body fluids in numerous human in situ studies. **(Reynolds.E.C)**[36]**,** ***G.D.Walker (2009)***[51] and ***Lijima Y (2004)***[25]**.**

Although CPP-ACP paste shows promising results in early sub-surface lesions, some studies concluded that as the lesion progresses it is not effective in prevention or progression the lesion **Shirahatti R et al 2007**[43], **Lata et al 2010**[23]. **Clarkson 1991**[6] who suggested that the presence of soluble phosphoproteins from dentin inhibited remineralization.

Clinpro™ Tooth Crème with 0.21% w/w Sodium Fluoride Anti-Cavity Paste is a white creme that contains 950 ppm fluoride and an innovative Tri-calcium phosphate ingredient and was recently introduced by 3M ESPE. This contains a fluoride compatible functionalized calcium phosphate ingredient and imparts superior remineralization at both the enamel surface and within the subsurface lesion ***(Karlinsey et al., 2009)***[19]**.**

Many studies have shown that combinations of calcium and fluoride can significantly boost remineralization relative to either mineral alone ***(Karlinsey et al., 2009***[19]***; Reynolds, 2008***[38]***).*** However, the ability to formulate a dentifrice with both bioavailable calcium and fluoride has remained elusive due to the rapid reaction kinetics that lead to premature calcium fluoride formation within the dentifrice tube. The functionalized TCP technology solves this problem by protecting its bioavailable calcium with a

fluoride-repelling surfactant (sodium lauryl sulfate) and as a result, can be readily combined in an aqueous dentifrice formulation with sodium fluoride. These results suggest that the synergistic combination of fluoride plus fTCP may provide superior dental health benefits over a dentifrice system and is designed to promote faster dispersion and therefore greater fluoride uptake, into enamel white-spot lesions[19,20].

The evidence base for TCP with 0.21% w/w Sodium Fluoride (CLINPRO-functionalized tri-calcium phosphate) is limited to laboratory studies and human in situ and clinical trial data to support their use in inadequate. Hence it requires sufficient research to validate its effectiveness.

In view of the above considerations, the present in-vitro study aimed to investigate the remineralization potential of topical application of CPP-ACP (GC Tooth Mousse) and TCP with 0.21% w/w Sodium Fluoride (Clinpro Tooth Crème) on artificially induced sub surface lesions in human primary and permanent teeth.

Twenty human permanent and twenty human primary teeth were collected. All the teeth were coated with an acid resistant nail varnish (Revlon, USA) leaving two narrow rectangular windows (occlusal 1/3 and gingival 1/3) of approximately 1mm size, on the intact buccal surface. Teeth were immersed in demineralizing solution for 96 hrs to create artificial sub-surface caries lesions[47,22].

After demineralization the gingival window on each teeth was closed with nail varnish which serves as a demineralization control.

The samples from permanent teeth (n=20) were then randomly assigned to two groups I (CPP-ACP Paste) and II (TCP Clinpro Paste) and primary teeth (n=20) were then randomly assigned to two groups III (CPP-ACP Paste) and IV (TCP Clinpro Paste) based on the treatment agents applied

Topical application of the remineralizing agents (GC Tooth Mousse and Clinpro) was carried out for three minutes twice daily followed by immersing in remineralizing solution for 10 days in permanent teeth and 7 days in primary teeth. The solution concentration and pH were maintained in the range reported to exist in oral fluids. To avoid the risk of solutions becoming saturated, fresh remineralizing solutions were changed daily[17,32,48].

Scanning electron microscope was used to analyse the remineralization changes qualitatively[15,16,29,30,34]. The specimens were analyzed and photomicrographs of the enamel surface were taken at 1500 X magnification.

The photomicrographs of sound enamel surface prior to demineralization procedure in all groups appeared to be relatively smooth and it is characterized by a faint, ripple-like imbrication lines.

The demineralised enamel surface in all groups resembled honeycombed pattern and transitional stages of surface breakdown from pit to porosity was seen as demineralization progressed.

The photomicrographs taken after remineralisation of enamel surface appeared almost the same as the photomicrographs of sound enamel and were characterized by faint, ripple-like configurations.

The changes in mineralization were done quantitatively by EDAX[15,34]. It is a micro analytical technique that is employed to estimate quantitatively the amounts of mineral in a given tooth sample. The digital outputs of the EDAX values were interpreted numerically as Ca/P ratios. Data were presented as means and standard deviation values. ANOVA Analysis was used to compare between means of the groups. Post-hoc test was used to determine significant differences between the means.

The effect of remineralizing agents was evaluated from the EDAX Calcium and Phosphorous values. They were converted into Ca/P ratio of study groups and are represented in Table [I - IV]. The Ca/P ratio of sound enamel of several enamel sections measured by EDAX for permanent teeth was 1.928 (mean value), which is consistent with previous determinations of MithraNHegde **2007**[15] (with mean Ca/P values 1.770). The Ca/P ratio of the enamel in the demineralized lesion had fallen to 1.645 (mean value), suggesting some precipitation of an acidic calcium phosphate phase from the lesion during demineralization. Remineralization of the lesions with treatments CPP-ACP (GC TOOTH MOUSSE) and TCP with 0.21% w/w Sodium Fluoride (CLINPRO) in permanent teeth resulted in an increase in the Ca/P ratio to 1.759 and 1.725 respectively. This increase in the Ca/P ratio

observed in the partially remineralized lesions is consistent with the deposited mineral being the thermodynamically most stable form, hydroxyapatite. The present study findings were similar to that seen by ***REYNOLDS E.C (1997)***[34] (1.63 with CPP-ACP).

The Ca/P ratio of sound enamel in primary teeth was 1.843 (mean value), after demineralization it was 1.459 (mean value) and after remineralization 1.676 in CPP-ACP and 1.623 in TCP 0.21% w/w Sodium Fluoride.

Remineralization effect of two pastes in primary and permanent teeth were (Table V) represented as mean remineralization change 0.105 ± 0.0512 (Group I), 0.088± 0.0330 (Group II), 0.214± 0.0232 (Group III), 0.170± 0.0290 (Group IV).

The results of present study showed remineralization change with CPP-ACP in permanent teeth (***0.105)*** was better than the findings of ***REYNOLDS E.C 1997 (0.08)***[34] and lesser than **Mithra N Hegde 2007 (0.21)**[15].

The present study showed a mean remineralization change for TCP was ***0.088*** in Permanent teeth similar results were obtained by ***Karlinsey L Robert 2009***[19] with TCP who showed 30% denser subsurface lesions when determined by vicker's hardness testing.

The present study showed a mean remineralization change with CPP-ACP **0.214** in Primary teeth, which were similar to the study done by

BADR Y SHERINE 2010[3], on comparative evaluation of CPP-ACP, APF gel, Na F varnish found that surface hardness increased 5% after treatment with CPP-ACP group, 4.6% with Fluoride varnish and 10.6% with APF gel.

The present study showed a mean remineralization change for TCP with 0.21% w/w Sodium Fluoride was ***0.170*** in Primary teeth. There are no known studies reporting the efficacy of TCP with 0.21% w/w Sodium Fluoride (CLINPRO TOOTH CRÈME) in primary teeth indicating the requirements of more studies and clinical trials to evaluate its efficacy.

On comparison of remineralization effect of two pastes in permanent teeth (shown in Table VI) CPP-ACP (Group I) 0.105 ± 0.0512 showed better effect than TCP with 0.21% w/w Sodium Fluoride (Group II) 0.088± 0.0330, which was not statistically significant(P=0.389).

Similar results were obtained in the study done by **VLN Kumar, 2008**[22] where there was a decrease in lesion depth upto 10.1 % with CPP-ACP compared to fluoride formulations. In a study by **BADR *Y SHERINE 2010***[3] surface hardness increased upto 13.1% with CPP-ACP, 6.7% with Fluoride varnish and 6.3% with APF gel.

CPP-ACP (Group III) 0.214± 0.0232 showed better effect than TCP with 0.21% w/w Sodium Fluoride (Group IV) 0.170± 0.0290 in *Primary teeth* which was statistically significant (P=0.001).

The results of our study showed that both the remineralizing agents were able to remineralize sub surface artificial caries like lesions both in

primary and permanent teeth, but the effect was more significant in primary teeth. Comparing primary and permanent teeth, in primary teeth there was more amount of demineralization seen when compared to permanent teeth. This can be attributed to the fact that, there is structural difference between both primary and permanent enamel[3,53]. Deciduous teeth demonstrate a higher degree of enamel porosity and a lower degree of mineralization than permanent teeth. This was attributed to greater density of the interprismatic fraction and the prism-junction in deciduous enamel than its permanent analogue. This difference in porosity might contribute, at least in part, to the observed variation to the response to various protective agents.

Other differences between deciduous and permanent tissues may also be of importance. Primary enamel has less organized microcrystals and a greater diffusion coefficient. Furthermore, primary teeth possesses an aprismatic layer on its outer surface, which erodes in a highly irregular manner and is probably not as liable to erosive destruction when compared to prismatic enamel[3] .

In the present study CPP-ACP and TCP showed certain amount of remineralization potential on artificial caries – like subsurface lesions in primary and permanent teeth. The remineralizing efficacy of CPP-ACP was better compared to TCP with 0.21% w/w Sodium Fluoride but the effect was more significant in primary teeth (P=0.001) and non-significant in permanent teeth (P=0.389). Since it is an *in vitro* model used with small sample size, the

results should be substantiated by longitudinal caries incidence studies and *in situ* studies Different results may be expected in an *in situ* or *in vivo* situation where CPP-ACP can bind to oral bacteria (***Rose RK***)[40] and be released from oral reservoir (***Schupbach P***)[41].

CONCLUSION

1. Both CPP-ACP (casein phosphopeptide amorphous calcium phosphate paste) and TCP with 0.21% w/w Sodium Fluoride (Tri-Calcium Phosphate) showed remineralization potential on artificial caries – like subsurface lesions in primary and permanent teeth.

2. A comparative evaluation showed better remineralization effect of CPP-ACP (GC Tooth Mousse) than TCP with 0.21% w/w Sodium Fluoride (Clinpro Tooth Crème) both in primary and permanent teeth, but the effect was more significant in primary teeth (P=0.001) and non-significant in permanent teeth (P=0.389).

These remineralizing agents can be prescribed for children who are more susceptible to caries, which can help to remineralize early enamel lesions and also protect the teeth from further caries challenge.

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SUMMARY

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This in-vitro study was designed to investigate the effect of CPP-ACP (GC tooth mousse) and TCP with 0.21% w/w sodium fluoride anti-cavity paste (clinpro tooth crème) on remineralization of artificial caries – like subsurface lesions in primary and permanent teeth.

Twenty human permanent and twenty primary teeth were collected. All the teeth were coated with an acid resistant nail varnish (Revlon, USA) leaving two narrow rectangular windows (occlusal 1/3 and gingival 1/3) of approximately 1mm size, on the intact buccal surface. Then immersed in demineralizing solution for 96 hrs to create artificial sub-surface caries lesions. The samples from permanent teeth (n=20) were then randomly assigned to two Groups I and II and primary teeth (n=20) were then randomly assigned to two Groups III and IV based on the treatment agents applied.

GROUP I - CPP-ACP (GC Tooth mousse) in permanent teeth

GROUP II- TCP with 0.21%w/w sodium fluoride (CLINPRO) in Permanent teeth

GROUP III- CPP-ACP (GC Tooth mousse) in Primary teeth

GROUP IV- TCP with 0.21%w/w sodium fluoride (CLINPRO) in Primary teeth

Topical application of the remineralizing agents (GC Tooth Mousse and Clinpro) was carried out for three minutes twice daily followed by immersing in remineralizing solution for 10 days in permanent teeth and

7 days in primary teeth. The solution concentration and pH were maintained in the range reported to exist in oral fluids. To avoid the risk of solutions becoming saturated, fresh remineralizing solutions were changed daily.

After the treatment period, the mineral content was quantified using EDAX & surface morphology was analyzed under SEM. The values were statistically analyzed.

The effect of remineralizing agents was evaluated from the EDAX Calcium and Phosphorous values. They were converted into Ca/P ratio of study groups and are represented in Table [I - IV]

Remineralization effect of two pastes were compared and shown in Table [VI]. On Comparison of Ca/P Ratios CPP-ACP (Group I) 0.105 ± 0.0512 showed better remineralizing efficacy than TCP with 0.21% w/w Sodium Fluoride (Group II) 0.088± 0.0330 in *Permanent teeth*, which was not statistically significant(P=0.389).

On Comparison of Ca/P Ratios CPP-ACP (Group III) 0.214± 0.0232 showed better remineralizing effect than TCP with 0.21% w/w Sodium Fluoride (Group IV) 0.170± 0.0290 in *Primary teeth* which was statistically significant. (P=0.001).

-Both CPP-ACP (GC TOOTH MOUSSE) and TCP with 0.21% w/w Sodium Fluoride (CLINPRO) showed remineralization capacity, with CPP-ACP showing better remineralization capacity when compared to TCP with significant effect in primary teeth.

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